Physico-chemical properties and microstructure of Dangke cheese by inoculated of *lactococcus lactis* at different temperature storage

Ratmawati Malaka
Syahriana Syabil
Fatma Maruddin

Laboratory of Biotechnology of milk Processing
Faculty of Animal Science, Hasanuddin University, Indonesia

Keywords
Dangke, duration of ripened, *Lactococcus lactis*, physicochemical properties, micro-structural properties.

Abstract
Dangke which is a traditional cheese Enrekang South Sulawesi has self life only a few days and have a quality that is still often varied and less good. Dangke quality can be improved with the ripening process. *Lactococcus lactis* is usually used as a starter in the manufacture of cheese ripening, including Dangke (traditional cheese of Indonesia). The purpose of this study is to improve the quality of dangke by ripening and inoculation of starter culture bacteria to obtain fermented dangke as other types of fermentation cheese. The changes in physicochemical properties and microstructure of Dangke cheese made from cow fresh milk, coagulated with papaya sap and inoculated by *Lactococcus lactis* were investigated during 0 - 6 days ripening period. Physicochemical data were statistically processed by analysis of variance followed by the least significant difference test, while the micro data processed descriptively. The results showed that the ripening time indicates that provide an opportunity for *L. lactis* to continue to be active on carbohydrate metabolism, resulting in a decrease in lactose, and pH. Results also showed that the moisture and fat content decreased, lactic acid and total protein increased. Microstructure observations indicate that the fat globules varying magnitude and matrix proteins are not evenly distributed but look more compact in dangke with the maturation of six days.

Introduction
Cheese is a milk protein or casein clot through the addition of acid or enzymes or fermentation by lactic acid bacteria (LAB), which is separated from milk serum or whey (Malaka et al., 2003). Cheese is a dairy product that is in demand by today's consumers. Cheese is very diverse kind in the market. It is an excellent dietary source of high-quality protein, vitamins and minerals such as absorbable dietary calcium. Their styles, textures and flavors depend on the origin of milk, animal’s diet, butterfat content, bacteria and mold, the processing, and aging condition (Fox, et al., 1996; Miller et al., 2007). The ripening process is one factor that determines the type of cheese that exists. The cheese was un-ripening which a local product of South Sulawesi named dangke.

Dangke is a type of fresh cheese made from buffalo milk, cow, goat or sheep latex is coagulated with papaya (Carica papaya) or pineapple juice (Malaka, 2014; Fatma et al., 2013; Hatta et al., 2013; Malaka et al., 2017a; Malaka et al., 2017b). Dangke contains 47.75% of water, 33.89% of fat, 17.01% of protein and other components in small quantities of vitamins and minerals (Marzoeki, 1978). Self life of Dangke at room temperature storage is only about 2 days at room temperature (Hatta et al., 2013). Dangke is a type of traditional cheese, semisolid cheese as available in the traditional market and traditionally manufactured by lokal people (Rasbawati et al., 2014). Dangke quality can be improved with the ripening process. *Lactococcus lactis* can be used as a starter in the manufacture dangke ripening. According Pato (2003) that the LAB is widely used in the manufacture of cheese ripening is a genus *Lactococcus, Lactobacillus, Streptococcus* and *Leuconostoc*. *Lactococcus lactis* has been widely used as starter cultures in fermented milk in the worldwide. *Lactococcus lactis* used to produce food products such as cheese, kefir, sour cream and buttermilk.

*Lactococcus lactis* is a Gram-positive, spore-forming and are not motile. The Role of *L. lactis* in cheese making is to produce acid and flavor, helps the formation of curds and produce lipolytic and proteolytic enzymes that are important in cheese ripening (Malaka, 2014). *Lactococcus lactis* is also
producing endocellular proteinase enzymes released when the cells undergo autolysis to change the texture and affect the aroma and flavor of the cheese (Bachrudin et al., 2000).

*Lactococcus lactis* is role in the preservation of organic produce products with acid, bacteriocins and hydrogen peroxide. *Lactococcus lactis* in order to work properly requires proper growth environment. Optimal growth will affect the ripening of cheese that can be done at a temperature of 15-16°C for 15-30 days or at 5 °C for 2-4 weeks. Malaka (2014) suggested that the cheese maturation process occurs in storage during the period and a certain temperature. The purpose of maturation is to provide an optimal opportunity for Lactic Acid Bacteria to change the composition of the cheese resulting in flavor, aroma and texture specific (typical).

*Lactococcus lactis* starter culture can optimally enhance the physicochemical properties and microstructure dangke ripening. *Lactococcus lactis* fermentation of carbohydrates is to produce lactic acid. The amount of lactic acid decreases the value of acidity (pH) of the product so as to form clotting protein (casein) which causes the formation of curd dangke. The more protein clotted that will cause the cheese dangke more firm and harder and decreases humidity.

The release of water content in the cheese making process will affect the microstructure dangke. Microstructure describes the position of casein and density deployment of the fat globules in dangke. Different ripening time determines the metabolism of *L. lactis* and the nature of the product is also different. The purpose of this study was to analyze the effect of the ripening period on the physicochemical properties and microstructure dangke with the inoculation of *L. lactis*.

**Materials and methods**

**Materials and Experimental design**

Raw fresh cow’s milk was used in the manufacture of cheese samples. Powdered crude papain (1/4000, produced in milk processing biotechnology laboratory, Hasanuddin University, Indonesia). *Lactococcus lactis* subsp. *lactis* FNCC-0086 was obtained from the Center for Food and Nutrition Studies at Gadjah Mada University in Yogyakarta. Fresh raw milk supplied from Sintari milk cooperative, Gunung Perak Village, Sinjai District, South Sulawesi, Indonesia.

This study was carried out experimentally using a completely randomized design (CRD) 3 treatments (0 day, 3 days and 6 days) with 3 repetitions. The first method is the multiplication of the starter culture in accordance with the modified method of Malaka and Sulmiyati (2010). Full cream milk was dissolved 10% (w/v) and sterilized at 105°C for 5 minutes. The reconstituted skim milk was cooled to a temperature of 40°C, inoculated with starter culture as much as 3% and incubated at 37°C for 36 hours. To confirm that starter culture was active, and then RSM was propagating twice before being used as a starter in cheese making modifications.

In the Dangke making was used coagulant from papaya latex. Papaya latex obtained by collecting the sap of papaya, where sliced with a knife, the collected crude papain was drying for 50 hours in freeze dryer (method Chaiwut et al., 2007; Deulgaonkar and Thorat 2008, where this method modified by Fatma et al., 2012). Crude papain then reconstituted with sterile distilled water of 1: 100 (w / v) before used in research.

**Dangke Making**

Dangke manufacture is modified from Irfan research (2016). Fresh milk (500 ml) after heating to 40°C for 10 mim was added a solution of papain as much as 0.2%. The heating temperature increased to 95°C and maintained at this temperature for 5 minutes. After protein coagulates milk, the milk is left until the temperature reaches 70°C, then the separation of the whey by filtration using gauze and draining for 15 min). Curd that formed and given a pressure of 2 kg for 3 minutes, and left in the mold for 20 minutes to maximize the separation of whey.

Dangke was added starter culture as much as 1% and then was ripened at a temperature of 5°C for 0, 3 and 6 days. Independent variable tested was the acidity (pH) (Fardiaz, 1993), the water content (AOAC, 1995), the protein (AOAC, 1995), the carbohydrate (AOAC, 1995), the lactic acid (AOAC, 2005) and the fat content (AOAC, 1995). The microstructure evaluation was use light microscopy to monitor the changes and interactions that occur during the formation of the milk gel with hematoxylin eosin staining.
techniques (method by Ohashi et al., 1978; Ohashi et al., 1983 as modified by Malaka (1997). The experimental design of Dangke is detailed in Table 1.

Table 1. Experimental design of white cheeses

<table>
<thead>
<tr>
<th>Dangke Type</th>
<th>Starter culture</th>
<th>Papain</th>
<th>Ripening and storage (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dangke with pasteurization at 95°C, 5 min</td>
<td>1 %</td>
<td>0.2%</td>
<td>0</td>
</tr>
<tr>
<td>Dangke with pasteurization at 95°C, 5 min</td>
<td>1 %</td>
<td>0.2%</td>
<td>3</td>
</tr>
<tr>
<td>Dangke with pasteurization at 95°C, 5 min</td>
<td>1 %</td>
<td>0.2%</td>
<td>6</td>
</tr>
</tbody>
</table>

The following analyses were performed on the cheese samples: pH was determined by pH-meter; titratable acidity (% lactic acid) was determined by titrating 10 g of sample with 0.25 N NaOH to a pink endpoint using a phenolphthalein indicator. The fat composition determined by the Gerber method. Protein was determined by Kjeldahl method (Scancalopore and Longone, 1988; IDF, 1993).

Data Analysis

Dangke samples data was determined toward physiochemical properties (pH, titratable acidity and the chemical composition of product). Physicochemical testing data were analyzed using analysis of variance. If the analysis shows a significant effect, the analysis followed by Duncan’s multiple range test (Gasperz, 1994). Micro-structural observation data were analyzed descriptively and compared with the existing literature on the various studies.

Results and discussion

Dangke which ripened cheese is an innovation of traditional South Sulawesi through inoculation technologies fermented milk starter cultures. Cheese is classical dairy product, which is strongly judged by its appearance and texture; hence, a renewed interest in its microstructure has been in the rise, as sophisticated techniques of analysis become more and more informative and widely available (Pereira et al., 2009). This fermentation technology using *Lactococcus lactis* isolated from Dangke itself. *Lactococcus lactis* requires optimal temperatures to obtain the expected quality dangke as a type of cheese ripening. This study treatment was use ripening time for 0, 3 and 6 days. Long maturation is expected to provide the specific nature of the product, which is indicated on the physico-chemical properties and microstructure.

The results showed that the physical properties of the observed dangke ripening not different in form with natural Dangke produced by the home made industry. Microstructure of dangke looks more compact three-dimensional network of casein with fat evenly distributed in the matrix. The physico-chemical properties of dangke cheese during ripening by *L. lactis* are presented in Table 1.

Table 1. Physico-chemical properties of dangke at several of times of ripening

<table>
<thead>
<tr>
<th>Physico-chemical properties</th>
<th>ripening time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>24.3±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactic acid (%)</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>pH</td>
<td>5.3±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>12.4±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water (%)</td>
<td>60.4±1.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>20.71±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: <sup>abc</sup>-superscript who follow the average value in the same row indicate significant differences (P<0.05).

Results of analysis of variance showed that the ripening times was highly significant (P<0.01) on carbohydrate, pH, protein, fat and moisture content of dangke. Levels of carbohydrate were decreases average of 2.7 to 2.9% along the length of ripening time, occurs because of the activity of the breakdown of carbohydrates into lactic acid by *L. lactis*. This is in accordance with the study of Widodo (2003) which states that the starter is often used in cheese ripening is *L. lactis*, will metabolize carbohydrates into lactic acid during ripening.

Time of ripening was no significant effect (P> 0.05) on total of lactic acid of dangke. Decreased levels of carbohydrates are not entirely because of the metabolic processes of *L. lactis* into lactic acid, but
may be also of metabolic factors be caused due to owned by L. lactis on exopolysaccharide production. Lactic Acid Bacteria have the ability to produce exopolysaccharide in the form of slime (Malaka, 2014, Malaka, 2005; Malaka, 2003, Malaka, 2007).

Further test results for significance different test showed that the pH, carbohydrate, protein, moisture and fat content dangke showed differences (P <0.05) between each ripening time. Lactococcus lactis survival by using energy from the metabolism of organic component of milk so that when the availability of carbohydrates runs out the bacteria will be dying.

Low levels of carbohydrates in ripened dangke shows the success of the ripening process. This is in accordance with the opinion of Gaman and Sherrington (1992) which states that foods such as cheese and butter made from milk usually contains no carbohydrates, except for soft cottage cheese contains very little carbohydrates. Carbohydrate metabolism in dangke will produce lactic acid.

Determination of acidity in milk and milk product based on the total lactic acid is one method of determining the level of acidity that influenced by many factors. According to result of study by Malaka and Sulmiyati (2010) that total of lactic acid was increased by increasing the ripening time of cheese. Total of lactic acid in ripened dangke closely related to the type of lactic acid bacteria used (Hadiwiyoto, 1983). Malaka (2010) which states that the acidity of the milk and milk product also can be caused by phosphoric acid, citric acid, casein and albumin properties as well as the presence of CO2 in the milk.

The lactic acid formed during ripening will be increase and pH will decline. These results are in accordance with the opinion of Hidayat et. al. (2006) which states that during ripened or maturation will occur as a result of biochemical processes in bacterial activity that will be formed as a result of lactic acid fermentation process that occurs which is characterized by a decrease in pH.

When the pH value decreased then the point isoelectric on dangke protein was resulting in coagulation. One of the primary activities that occur during the ripening process is proteolysis, thus affecting the protein component dangke. This is in accordance with Widodo (2003) which states that the lactic acid is formed from the metabolic processes of lactose during cheese ripening where L. lactis as a source of energy for biosynthesis of cell proteins and other macromolecules.

Dangke protein content is the result of proteolysis by L. lactis and milk enzymes. During the ripening process to form lactic acid was characterized by a decrease in pH in order to reach the isoelectric point of the protein. Malaka (2014) states that the lactic acid molecules that are positively charged intersect with milk proteins negatively charged neutralization will occur that causes coagulation of proteins (settles). Proteins coagulation will continue to happen as long as lactic acid is formed. This is in accordance of Rahman et al. (1992) which states that during ripening will occur perfecting the process of cheese making. Milk proteins are insoluble and will be dissolved at the time of ripening by enzymes and bacteria activity.

In the other of the formation of acid by L. lactis, dangke protein is also addition by the composition of the body of the bacterium itself, because of the composition of the bacterial body is protein. This is supported by Gaman and Sherrington (1992) which states that the microorganism has high protein content and has potential as a valuable source of protein. Furthermore the protein content of ripened dangke can be decreased and increased in accordance with the ability of bacteria to grow.

Ripening and duration time affects the growth phase of L. lactis and proteolysis results. The protein content is also influenced by proteolytic activity during Dangke processing. The protein content dangke with ripening 6 days was the highest protein content. Protein content (24.54%) on dangke ripened can be classified as the standards for class ripened cheese. This is in accordance with Kusnandar (2010) which states that cheddar cheese contains protein 24.9%. Gaman and Sherrington (1992) was states that the protein content of cheddar cheese was 26%.

Lactococcus lactis perform lipolysis and proteolys. Kusnandar (2010) states that lipolysis is a reaction to the release of free fatty acids from the glycerol in fat structure. Then Lipolysis also occurs because of the lipase activity of milk of dangke. Rahman et al. (1992) declare that the caseins bind approximately 50% of the total content of bound water (moisture). Kusnandar (2010) which state that the clotting properties of the casein in cheese making would eliminate the ability of the water holding capacity, so the physical and chemical properties of water in food are different because the water bound
in food with degrees or bond strengths was variety. Water in food can be found intercellular between cells, trapped in cells or bound to a chemical compound in food.

Metabolic processes by *L. lactis* will be decreased by the decreasing ability to grow, because the availability of lactose depleted and the total acid formed is too high. Rahman et al. (1992) which states that culture will continue to produce lactic acid until the growth of bacteria was decreased, because of the number of cells death increased if the acidity is too high. Gorbach (2001) was states that the growth of *L. lactis* temperature is 5-50ºC and the optimum growth at temperature of 37ºC.

Protein neutralization process will be liberating water molecules so that the protein is getting crowded and the water content in food has declined. Water levels will continue to decline during ripening because the lactic acid that keeps this process. Kusnandar (2010) which states that water molecules can be chemically bonded to the other molecules through hydrogen bonds and can be in food hydrophilic surface forming layer (monolayer and multilayer).

*Lactococcus lactis* growth activity was determining the ability to produce enzymes and perform hydrolysis of fats. Fat content influenced by the hydrolysis process and the fat content of raw materials dangke. The dangke lowest fat content was result has ripening during 6 days (8.03%) where the fat content was approaching (3-4%). Low levels of fat of ripened dangke can also be influenced during the process of whey syneresis. Low fat and high protein content are special characteristic of ripened dangke dairy products.

Physico-chemical properties dangke ripened illustrated in cross section microstructure. The results of the study (Figure 1.) appear the little balls are huge numbers and this is a collection of globules of fat and casein. Ripening process by *Lactococcus lactis* provides an opportunity to convert carbohydrates into lactic acid milk. The lactic acid formed will help the clotting process dangke protein, furthermore protein will be more solid and fat globules appear more clearly. Physico-chemical properties dangke ripened illustrated in cross section microstructure. The results of the study (Figure 1.) appear the little balls are huge numbers and this is a collection of globules of fat and casein. Ripening process by *Lactococcus lactis* provides an opportunity to convert carbohydrates into lactic acid milk. The lactic acid formed will help the clotting process dangke protein, furthermore protein will be more solid and fat globules appear more clearly.

Figure 1. Figure microstructure dangke using hematoxylin eosin staining magnification 100X
A. Ripening 0 days
B. Ripening 3 days
C. Ripening 6 days

Six days long of ripening have shown clearly fat globules with the smallest cross section and increasingly dense protein matrix. The process was result a solid matrix that connects the fat globules with protein matrix. Anderson and Mistry (1993) states that tridemential matrix of typical microstructure of cheese is a structure filled most of the matrix protein with a little amount of fat globules dispersed in
the matrix. Tamime et al. (1990) studied the processing of Cheddar cheese with skim milk powder, and its effect upon the microstructure of the final product.

Conclusion
The during ripening was increases lactic acid and protein levels, decreases of carbohydrates, pH, moisture and fat content, furthermore as well as result a more compact of microstructure of Dangke. Ripening temperature 5°C with 6 days of ripening time indicates the best nature dangke.

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